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Expression levels of parvalbumins determine allergenicity of fish species

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beta-parvalbumin; cross-reactivity; fish allergy; food allergen; whiff.

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Abstract

Background: Parvalbumins are the most important fish allergens. Polysensitization to various fish species is frequently reported and linked to the cross-reactivity of their parvalbumins. Studies on cross-reactivity and its association to the allergenicity of purified natural parvalbumins from different fish species are still lacking. In addition, some studies indicate that dark muscled fish such as tuna are less allergenic.

Methods: Total protein extracts and purified parvalbumins from cod, whiff, and swordfish, all eaten frequently in Spain, were tested for their IgE-binding properties with 16 fish allergic patients' sera from Madrid. The extent of cross-reactivity of these parvalbumins was investigated by IgE ELISA inhibition assays. Additionally, the cDNA sequences of whiff and swordfish parvalbumins were determined.

Results: Extractable amounts of parvalbumins from cod were 20 times and from whiff 30 times higher than from swordfish. Parvalbumins were recognized by 94% of the patients in extracts of cod and whiff, but only by 60% in swordfish extracts. Nevertheless, a high cross-reactivity was determined for all purified parvalbumins by IgE inhibition. The amino acid sequence identities of the three parvalbumins were in a range of 62–74%.

Conclusions: The parvalbumins of cod, whiff and swordfish are highly cross-reactive. The high amino acid sequence identity among cod, whiff and swordfish parvalbumins results in the observed IgE cross-reactivity. The low allergenicity of swordfish is due to the low expression levels of its parvalbumin.

Especially in coastal countries, fish constitute an important part of the diet. In Norway allergy to fish is found in 0.1% of the population (1, 2). In a cross-sectional study carried out in Spain, fish allergy was diagnosed in 36 of 4991 patients (0,72%) referred to for allergy evaluation (3). Patients are often allergic to certain fish species while they tolerate others (4). In Spain, whiff, cod and swordfish are commonly consumed (5, 6). Allergic reactions to fish can be mild to severe (5, 7).

The major allergens of fish are parvalbumins besides minor allergens such as collagen and aldehyde phosphate dehydrogenase (8, 9). Parvalbumin is an acidic, calcium-binding 12 kDa protein resistant to heat and digestive enzymes (10–14). Fish muscles express multiple parvalbumin isoforms, which can be divided into two distinct evolutionary lineages. Although alpha-parvalbumins were described in fish, they are generally not allergenic (15–17). However, allergenic alpha-parvalbumins were reported in frog (18–21). Various IgE-reactive beta-parvalbumins of bony fish were described (10, 22, 23). Based on the high amino acid sequence identity of beta-parvalbumins from different fish, cross-reactivity among different fish species occurs frequently (4, 5, 22, 24–26).

Bony fish have fast twitching white muscle for rapid movements and dark muscle for continuous swimming. Active fish, such as tuna, skipjack (27), and swordfish have a higher proportion of dark muscles than bottom dwelling fish, such as cod, flounder (27), or whiff. Dark muscle contains lower levels of parvalbumins, thus these fish species are expected to be of lower allergenicity (27, 28).

In this study, we investigated the IgE cross-reactivity of purified parvalbumins from cod and whiff as examples for allergenic fish, and from swordfish as an example for dark muscled fish.

Material and methods

Protein extracts

Atlantic cod (*Gadus morhua*), swordfish (*Xiphias gladius*), and whiff (*Lepidorhombus whiffiagonis*) filets were purchased from local markets in Vienna, Austria, and Madrid, Spain. 500 g cod or 250 g whiff filets were extracted in three volumes (w/v) of 20 mM Bis-Tris, pH 6.5. After centrifugation at 17 000 ***g*** for 45 min at 4°C, the supernatants were filtered through Miracloth (Merck Biosciences, Nottingham, UK). As swordfish proteins precipitated in the extraction buffer used for cod and whiff, 80 g swordfish filet was extracted in double distilled water. The supernatant, obtained as described above, was dialyzed against 20 mM Bis-Tris, pH 7.0. Protein extracts were stored at 4°C.

Protein purification

All fish extracts were treated with 0.1% Biocryl BPA-1000 (Supelco, Bellefonte, PA, USA) to remove negatively charged nonprotein components such as nucleic acids. After centrifugation, the supernatants were applied to a DEAE Sepharose Fast Flow column (GE Healthcare, Little Chalfont, UK). Fractions containing parvalbumin were loaded onto a HiPrep 16/60 Sephacryl S-200 column (GE Healthcare). Cod or whiff parvalbumins were eluted as a single peak. For swordfish, a pool of fractions containing parvalbumin was dialyzed against 50 mM Na-acetate, pH 5.0 and purified by a SP-Sepharose Fast Flow column (GE Healthcare). The flow through was collected, dialyzed against 20 mM Tris–HCl, pH 8.0 and purified by anion exchange chromatography using a MonoQ 5/50 GL column (GE Healthcare).

Mass and sequence determination

The mass and the N-terminal sequence of whiff beta-parvalbumin was determined as described (23).

RNA isolation and cDNA synthesis

Total RNA of 60 mg whiff or 400 mg swordfish muscle tissue was extracted according to the RNeasy kit procedure (Qiagen, Hilden, Germany) and for quality testing the ratio $A_{260} : A_{280}$ was measured (whiff: 2.1, swordfish: 1.8). First strand cDNA was synthesized from 2 μg total RNA with an oligo-dT25 primer (5′-GGAGAAGGAT$_{25}$VN-3′), using MuLV reverse transcriptase (Fermentas, St Leon-Rot, Germany).

PCR amplification and sequencing

A fragment of whiff cDNA encoding beta-parvalbumin was amplified by PCR using oligo-dT$_{25}$ and the degenerate primer Wh1-fwd (5′-ATGACITTYGCIGGIYTIGAYGC-3′) designed on the basis of an internal amino acid sequence obtained by MS analysis. For swordfish, PCR amplification was carried out with the primer pair oligo-dT$_{25}$ and Sw1-fwd (5′-CTGAAGCTGTTCCTGCAGAAC-3′), which corresponded to the Chub mackerel parvalbumin sequence (EMBL: AB091470). In order to obtain the 5′ ends, 5′-RNA ligase-mediated rapid amplification of cDNA ends was performed using the GeneRacer Kit (Invitrogen, Carlsbad, CA, USA). For swordfish, a modified 5′-primer was used (5′-GAGCACGAGGACACTGAC-3′). 3′-primers for whiff parvalbumin were Wh2-rev (5′-CCTAACAAGGTCGGTGAACTC-3′) and Wh3-nested: (5′-GCCATCAACGTCACCGGCCTTCAG-3′) and for swordfish Sw2-rev (5′-CGCAGCCGCCTTGAAGTTCT-3′) and Sw3-nested: (5′-GTTCTGCAGGAACAGCTTCAG-3′). PCR products were gel-purified, cloned into the pCR2.1-TOPO vector (Invitrogen) and sequenced (IBL, Vienna, Austria). Sequences were aligned using AlignIR 2.0 (LI-COR Biosciences, Lincoln, NE, USA).

Sera and antibodies

Sera from 16 patients with clinical histories of IgE-mediated type 1 fish allergy were selected at the Allergy Department of the Hospital Clinico San Carlos (Table 1). Sensitization to fish was verified by determination of fish-specific IgE using ImmunoCAP (Phadia, Uppsala, Sweden) and skin prick testing to different fish species and *Anisakis simplex* (29). All patients were negative to *Anisakis*. Parvalbumins were detected by the mouse monoclonal anti-parvalbumin clone Parv-19 antibody (Sigma, St Louis, MO, USA) and rabbit polyclonal anti-Gad m 1 antibody (Tepnel BioSystems Ltd, Deeside, UK).

SDS-PAGE and immunoblotting

Total protein extracts and purified allergens were separated by SDS-PAGE under reducing conditions and either visualized by Coomassie Brilliant Blue or transferred to nitrocellulose membranes for immunodetection (23). In addition, blotted proteins were incubated with individual fish allergic patients' sera. Bound IgE was detected by ^{125}I-labelled rabbit anti-human IgE (MALT Allergy System Isotope Reagent, IBL Hamburg, Germany).

IgE ELISA and inhibition assays

Purified Gad m 1, whiff and swordfish parvalbumins (2 μg/ml) were coated to CovaLink NH plates (Nunc, Roskilde, Denmark). After blocking with Tris buffered saline, 0.5% Tween-20 (TBST), 3% (w/v) dry milk powder, plates were incubated with sera diluted in TBST 0.5% (w/v) BSA (P1, P2, P4, P14 diluted 1 : 10; P3, P8 diluted 1 : 7). Bound IgE was detected with an AP-conjugated mouse anti-human IgE antibody (BD-Biosciences Pharmingen, San Diego, CA, USA). Inhibition assays were performed by preincubating a serum pool with increasing concentrations of parvalbumins (1, 10, 20, 50, 100 μg/ml) or extracts (1 or 100 μg) and deter-

Table 1 Clinical and serologic characteristics of patients with type 1 fish allergy

					Cod		Gad m 1	Whiff		Lep w 1	Swordfish		Xip g 1
Patient	Age (years)	Sex	Fish-related symptoms	Total IgE (kU/l)	CAP class	CAP (kU/l)	ELISA (OD)	CAP class	CAP (kU/l)	ELISA (OD)	CAP class	CAP (kU/l)	ELISA (OD)
P1	11	m	AE, A	264.0	5	74.50	3.10	4	48.50	3.88	2	2.33	0.26
P2	5	m	U	33.5	3	8.91	0.77	3	14.20	1.08	2	1.16	0
P3	12	m	AE	422.0	3	9.60	1.02	3	6.06	1.43	2	1.02	0
P4	26	f	AN	1272.0	3	12.30	1.79	3	10.20	2.43	3	4.60	0.05
P5	36	m	OAS, U	1633.5	3	7.37	1.98	3	10.00	2.64	1	0.38	0.20
P6	35	f	U, AE	>2000	2	2.69	0.59	2	1.43	0.88	1	0.39	0.01
P7	9	m	U, V	56.3	3	17.20	2.49	3	11.30	3.19	3	4.32	0.75
P8	2	f	OAS	143.0	3	15.30	0.80	3	13.50	1.11	2	0.87	0.04
P9	30	f	AE, A, D	389.0	2	3.07	0.77	2	2.07	1.16	0	0	0.17
P10	34	f	AN	311.0	4	30.30	1.23	3	5.90	1.67	3	5.74	0.20
P11	21	f	U, AE	1724.0	4	45.10	0.98	4	19.90	1.32	3	9.70	0.04
P12	5	m	OAS	61.5	2	1.82	0.06	2	1.17	0.06	0	0	0
P13	1	f	AN	72.4	2	1.18	0.19	2	2.14	0.25	0	0	0
P14	3	m	OAS	450.0	4	47.50	3.58	5	83.80	3.62	3	10.90	0.34
P15	13	m	U	961.0	4	47.50	nd	4	23.20	nd	0	0	nd
P16	4	m	U	276.0	0	0	nd	0	0	nd	3	10.90	nd
Mean						20.27	1.38		15.83	1.76		3.29	0.15
SD						22.16	1.06		21.75	1.20		4.01	0.20

Mean, mean value; SD, standard deviation; m, male; f, female; A, asthma; AD, atopic dermatitis; AE, angioedema; AN, anaphylaxis; U, urticaria; D, dysphagia; V, vomiting; OAS, oral allergy syndrome; nd, not done.

mining residual IgE-binding to fish extracts. In order to control for IgE-binding to high molecular weight (HMW) allergens of cod and whiff, chromatographic fractions containing these proteins but no parvalbumins were used as inhibitors (1 or 100 μg/ml). Cross-reactivities between parvalbumins were determined with four patients' sera using purified parvalbumins (50 μg/ml) as inhibitors. Inhibition values are given as percent reduction of bound IgE compared with the controls where no inhibitor protein had been added.

Statistical analysis

Comparisons of ImmunoCAP results for the three fish species and of the IgE ELISA for the three parvalbumins were carried out by nonparametric tests for paired samples (Friedman and Wilcoxon tests). Paired correlations of the IgE responses to cod, whiff and swordfish whole extracts (CAP) with total IgE and parvalbumins (ELISA) were performed with the Spearman test. P-values < 0.05 were considered significant. Statistical analysis was carried out with SPSS (SPSS Inc., Chicago, IL, USA).

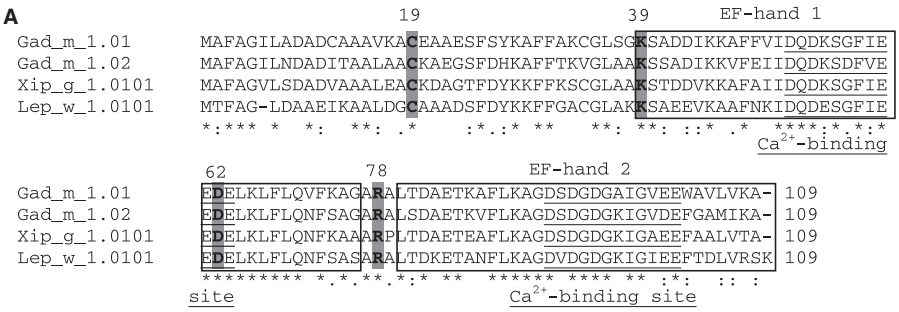
Results

cDNA cloning and sequencing of whiff and swordfish parvalbumins

The full-length cDNA sequence of whiff parvalbumin was obtained (EMBL: AM904681, designated Lep w 1.0101 by the IUIS allergen nomenclature sub-committee). It comprised 671 base pairs (bp) with an open reading frame (ORF) of 327 bp encoding a protein of 109 amino acids including the initiating methionine. The full-length cDNA of swordfish parvalbumin consisted of 692 bp with an ORF of 327 bp encoding a protein of 109 amino acids including the initiating methionine (FM202668, designated Xip g 1.0101). Both sequences contained conserved residues characteristic of beta-parvalbumins (Fig. 1, highlighted) (23, 30, 31). Theoretical isoelectric points were calculated as 4.5 for Lep w 1 and 4.43 for Xip g 1. The translated sequences were aligned with the published cod parvalbumin isoforms Gad m 1.01 (AM497927) and Gad m 1.02 (AM497928) (23) (Fig. 1A). All four parvalbumins shared sequence identities in the range of 62–74% (Fig. 1B).

Patients' characteristics

Clinical data of the patients are summarized in Table 1. All had a history of type 1 allergy to more than one fish species. The patients, 10 children (age 1–3 years) and 6 adults (age 21–36), showed moderate to severe symptoms. Seven patients could not remember the species involved in the allergic reaction. Of the remaining nine subjects eight reported symptoms with the intake of whiff and/or sole, and less frequently with other fish species such as hake, gilthead, anchovy, red mullet, tuna, swordfish or tope shark. All displayed positive skin prick test reactions to cod, whiff or swordfish. Fifteen patients (94%) had positive (≥ 0.35 kU/l) ImmunoCAP results to cod and whiff, 12 (75%) to swordfish. ImmunoCAP values to swordfish were significantly lower than those to cod ($P = 0.002$) and whiff ($P = 0.003$), whereas no significant differences were observed between whiff and cod



B

	Amino acid sequence identity (%)			
Allergens	Gad m 1.01	Gad m 1.02	Lep w 1.0101	Xip g 1.0101
Gad m 1.01	100	71	65	74
Gad m 1.02	71	100	62	71
Lep w 1.0101	65	62	100	69
Xip g 1.0101	74	71	69	100

Figure 1 (A) Protein sequence alignment of Gad m 1.01 (EMBL: AM497927), Gad m 1.02 (AM497928), Lep w 1 (AM9046811), and Xip g 1 (FM202668) generated by ClustalX, Version 1.83. Boxed: EF-hand 1 and 2, underlined: calcium-binding sites, highlighted: conserved amino acid residues characteristic of beta-parvalbumins, '*' indicate conserved, ':' highly conserved, '.' weakly conserved amino acid residues and '-' a gap. (B) Protein sequence identities.

ImmunoCAP values ($P = 0.12$). A strong and significant correlation ($P < 0.001$) was observed between ImmunoCAP values to cod and whiff ($r = 0.88$), whereas the correlations between ImmunoCAP values to cod and swordfish ($r = 0.37$) and ImmunoCAP values to whiff and swordfish ($r = 0.29$) were not significant ($P > 0.05$). No correlations were found between total IgE and CAPs to cod ($r = 0.17$, $P = 0.53$), whiff ($r = 0.07$, $P = 0.79$) and swordfish ($r = 0.10$, $P = 0.70$).

IgE recognition patterns to fish extracts

All 16 patients had IgE to at least one allergen of cod or whiff (Fig. 2). Fifteen patients (94%) recognized a 12 kDa protein (Fig. 2A,B), identified as parvalbumin by mono- and polyclonal anti-Gad m 1 antibodies (data not shown). In cod, additional IgE-reactive bands were observed at 30 kDa and around 40 kDa (Fig. 2A). In whiff extract, IgE-reactive proteins were detected at 17 kDa and in the HMW range (33–72 kDa) (Fig. 2B). Twelve of 16 patients showed IgE reactivity to parvalbumin in swordfish extract. One patient also had IgE to a 40 kDa protein in all fish extracts (Fig. 2C).

Purification and biochemical characterization of natural parvalbumins

Protein extracts of cod and whiff displayed similar protein patterns in SDS-PAGE with a prominent band at 12 kDa. The swordfish extract showed a remarkable amount of HMW proteins and two less prominent bands at 12 kDa (Fig. 3; lanes 1). The 12 kDa proteins were identified as parvalbumins in all extracts (data not shown).

Twenty milligrams parvalbumin from cod, 30 mg from whiff and 1 mg from swordfish were purified from 100 g fish muscle. The mouse monoclonal antibody detected one band for Gad m 1, Lep w 1 and Xip g 1 (Fig. 3; lanes 3). The polyclonal anti-parvalbumin antibody detected two isoforms of Gad m 1 and Xip g 1 (Fig. 3; lanes 5). A purity of >98% was determined for Gad m 1 and Lep w 1 by size exclusion chromatography (data not shown). Coomassie staining showed that Xip g 1 was >99% pure (Fig. 3; right panel, lane 2).

The identity of Lep w 1 was confirmed by N-terminal sequencing which revealed the first five amino acid residues as TFAGL. Mass spectrometric analysis of Lep w 1 revealed a single peak at 11624 Da, which is in agreement with the predicted theoretical mass of 11581.8 Da (data not shown). The difference of 42 Da is due to the acetylation of the N-terminus as previously reported for Gad m 1 (23). Mass spectrometric analysis confirmed the identity of the purified cod parvalbumin as Gad m 1 (data not shown) as described (23). In swordfish, two parvalbumin isoforms were identified by nanoLC-MSMS-based peptide mapping (data not shown). The lower molecular mass isoform was consistent with the cDNA sequence.

IgE reactivity of purified parvalbumins

IgE reactivity of the purified parvalbumins Gad m 1, Lep w 1 and Xip g 1 was confirmed by IgE ELISA using 14 patients' sera (Table 1). Gad m 1 and Lep w 1 were recognized by all patients in ELISA. The significantly highest amount of IgE was directed to Lep w 1 ($P = 0.001$). Ten patients' sera recognized the swordfish parvalbumin. The IgE level to Xip g 1 was significantly lower ($P = 0.001$) than those to Gad m 1 and Lep w 1. Strong and significant paired correlations ($P < 0.001$) were observed between the ELISA values to Gad m 1 and Lep w 1 ($r = 0.99$), Gad m 1 and

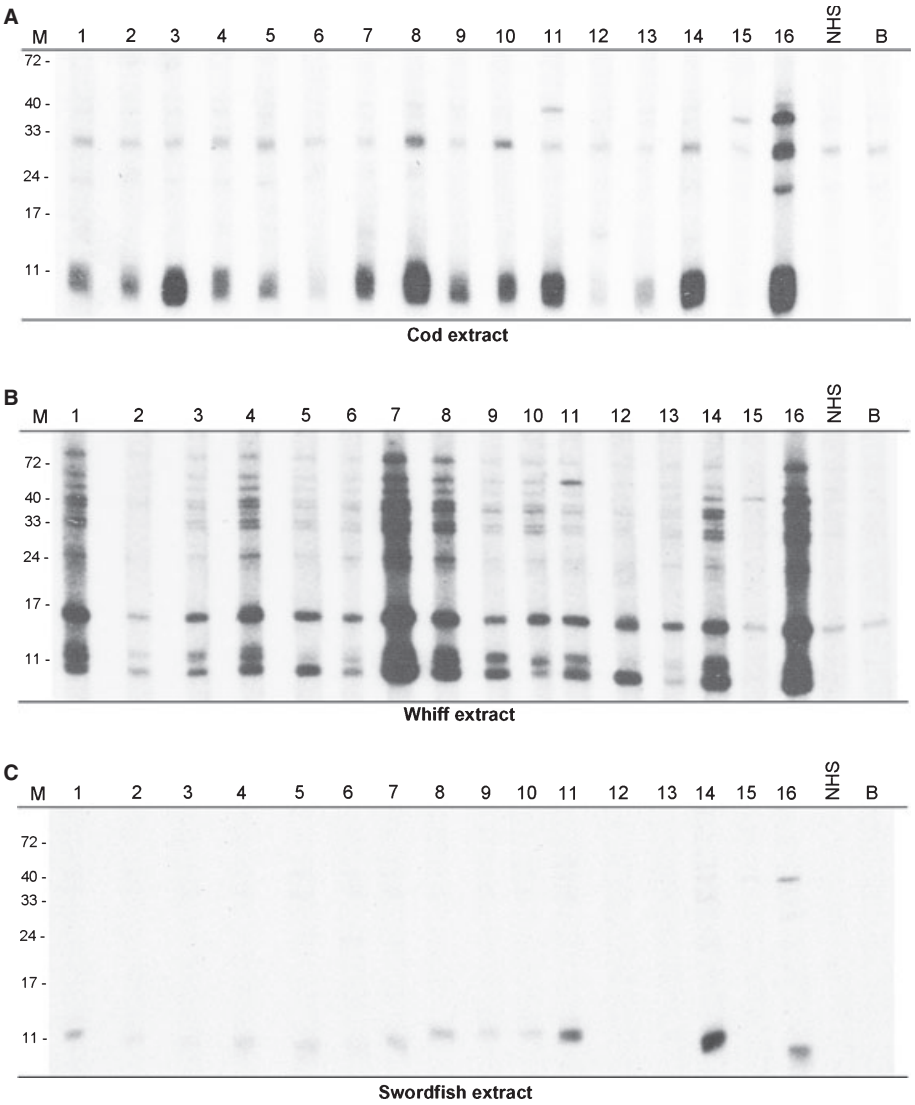


Figure 2 IgE-binding of fish allergic patients' sera to cod (A), whiff (B) and swordfish extract (C) was determined by IgE immunblotting. M, molecular weight marker (kDa); 1–16, sera of fish allergic patients; NHS, control serum; B, buffer control.

Xip g 1 ($r = 0.83$) and between Xip g 1 and Lep w 1 ($r = 0.85$).

Purified Gad m 1, Lep w 1 and Xip g 1 dose–dependently inhibited the IgE-binding of a serum pool (P1, 4, 8, 14) to protein extracts of cod, whiff and swordfish, respectively (Fig. 4, grey bars). Preincubation of the serum pool with 100 μg/ml Gad m 1 or Lep w 1 revealed an IgE inhibition of 89% and 92% to cod and whiff protein, respectively (Fig. 4, grey bars). IgE-binding was inhibited by HMW proteins of cod (38% at 100 μg/ml) and whiff (45% at 100 μg/ml) extracts (Fig. 4, white bars). IgE-binding to swordfish extract was inhibited by 84% after preincubation with 100 μg/ml Xip g 1 (Fig. 4, grey bars). Preincubation of the serum pool with fish extracts as positive control showed 100% inhibition (Fig. 4, black bars).

Gad m 1 was able to inhibit IgE-binding to immobilized Lep w 1 from 63% up to 97%. In contrast, sera preincubated with Lep w 1 were reduced in their IgE-binding to Gad m 1 by 42–75% (Table 2). A reduction of IgE-binding to Lep w 1 and Gad m 1 was achieved from 52% up to 85% using Xip g 1 as inhibitor. The IgE reactivity to Xip g 1 was reduced from 78 up to 100% by inhibition with Gad m 1 or Lep w 1. Lower inhibition of IgE-binding to whiff parvalbumin was determined for serum P3 preincubated with Gad m 1 (22%) or Xip g 1 (19%) (Table 2). Preincubation of serum P3 with Gad m 1 and Lep w 1 did not reduce the IgE-binding to Xip g 1. The values for the control inhibitions with Gad m 1, Lep w 1 and Xip g 1 were approximately 100% for all sera (Table 2).

Discussion

Fish allergic patients are often allergic to more than one species due to the cross-reactivity of their beta-parvalbumins (4, 32). However, differences in IgE reactivity to dark and white

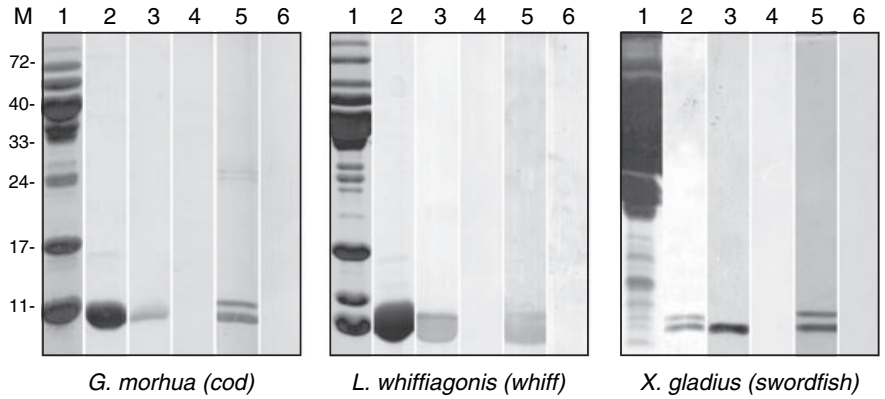


Figure 3 Purification and identification of parvalbumins. Fish protein extracts (Amounts loaded per lane – cod: 21 μg, whiff: 18 μg, swordfish: 60 μg) (lane 1) and purified parvalbumins (Amounts loaded per lane - Gad m 1: 12 μg of 1 mg/ml, Lep w 1: 24 μg of 2 mg/ml, Xip g 1: 6 μg of 0.5 mg/ml) were detected by Coomassie staining (lane 2) and immunoblotting with monoclonal (lane 3) and polyclonal (lane 5) antibodies, respectively. Lanes 4 and 6 show negative controls.

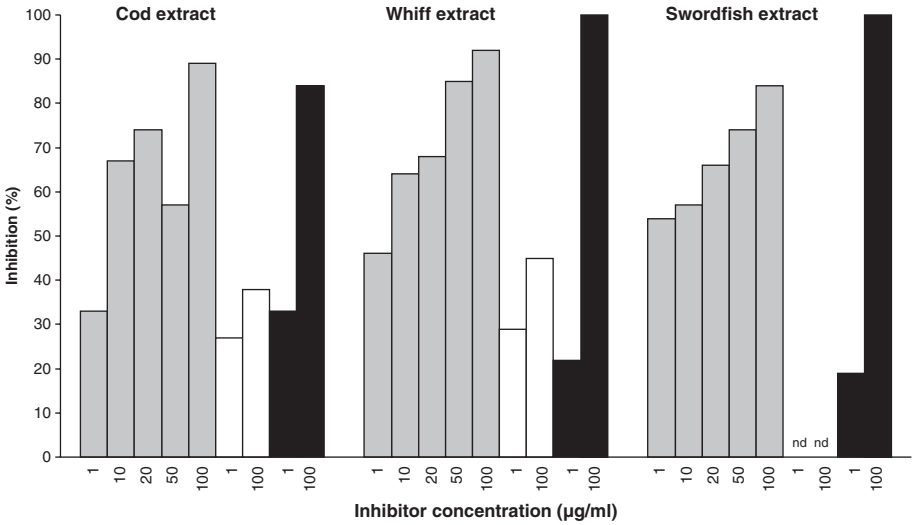


Figure 4 IgE ELISA inhibition assay with immobilized fish extracts. Grey: Purified parvalbumins Gad m 1, Lep w 1 and Xip g 1, respectively as inhibitors; white HMW proteins as inhibitors; black: fish extracts as inhibitors.

muscled fish have been reported (27). Active fish with a higher amount of dark muscles (27) were regarded as low allergenic (4).

In this study, we compared the IgE-binding of fish allergic patients' sera to cod, whiff and swordfish extracts and purified parvalbumins. We selected swordfish as a dark muscled fish and analyzed its IgE cross-reactivity with cod and whiff, both frequent causes of fish allergy in Spain (5). We describe for the first time the parvalbumins from whiff (Lep w 1) and swordfish (Xip g 1) as major allergens and report their cDNA sequences.

We established new purification protocols under native conditions for cod, whiff and swordfish parvalbumins to preserve calcium-binding which is important for their conformations (22, 23, 33). The low yield of swordfish parvalbumin (1 mg/100 g filet) compared to cod (20 mg/100 g) and whiff (30 mg/100 g) parvalbumins reflected the weak signals in IgE immunoblotting of the extract. We found only one parvalbumin isoform (Lep w 1.0101) in whiff. Two isoforms could be identified in cod and swordfish. Nearly complete inhibition of IgE-binding to cod and whiff extracts by purified Gad m 1 and Lep w 1 suggested that all IgE-reactive isoforms were purified. All patients' sera also recognized HMW proteins (Fig. 4, transparent bars), thus no complete inhibition could be obtained. In swordfish additional parvalbumin isoforms may be present. Only 84% inhibition of patients' IgE to this extract was achieved after incubation with purified Xip g 1.

IgE cross-reactivity of all studied parvalbumins was shown by IgE inhibition assays. The parvalbumins may share several identical IgE-binding epitopes, consistent with their high protein sequence identities (62–74%). We detected a lower reduction of IgE-binding to Gad m 1 after preincubation of sera

Table 2 IgE ELISA cross-inhibition of three fish parvalbumins

Coated	Gad m 1		Xip g 1		Lep w 1	
Inhibitor	Xip g 1	Lep w 1	Gad m 1	Lep w 1	Gad m 1	Xip g 1
Patients						
P1	85	75	91	78	68	64
P2	57	42	99	86	63	52
P3	81	60	0	0	22	19
P4	75	59	100	88	97	80

Control self inhibitions: Gad m 1 100%, Xip g 1 97%, Lep w 1 92%.

with Lep w 1 (in a range of 42–75%) than to Lep w 1 after preincubation with Gad m 1 (63–97%). These findings could indicate the presence of IgE reactive to species-specific IgE epitopes in cod and whiff parvalbumins. Patients might not have been sensitized by swordfish parvalbumin as IgE-binding to Xip g 1 was inhibited in a range of 80–100% with Gad m 1 and Lep w 1, respectively. Interestingly, patient P3's serum seemed to contain mainly species-specific IgE to the parvalbumin of the frequently eaten whiff. IgE-binding to Lep w 1 could only be reduced by around 20% using Gad m 1 and Xip g 1 as inhibitor (Table 2).

In previous studies, it was hypothesized that the degree of IgE-binding activity might be related to the amount of consumption (5, 34). As whiff is one of the most frequently consumed fish species in Spain, it is not unlikely that this patient was initially sensitized by Lep w 1. In our present work, all fish belonged to different taxonomic orders, but the protein sequence identities of parvalbumin isoforms were similar within the species and among fish species (around 70%) as already reported for Alaska pollock, Atlantic cod and salmon (7).

In conclusion, this study demonstrates the cross-reactivity among the parvalbumins of high allergenic cod and whiff, and the 'low allergenic' swordfish. High sequence identities support this finding. Parvalbumin was the major allergen in all studied fish including swordfish. The weak IgE-binding to swordfish parvalbumin was due to the low parvalbumin content of swordfish. We therefore suggest that cod or whiff parvalbumins are responsible for sensitization of fish allergic patients. We conclude that the low allergenicity of swordfish is due to the low expression level of its parvalbumin. Clinical studies involving oral challenges are currently ongoing to confirm its lower allergenicity in fish allergic patients.

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